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Attention: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

Gentlemen:

Enclosed is a copy of a Health and Safety Study that was just received from our parent company, Bayer AG.

We are submitting this study on behalf of Miles Inc., Mobay Road, Pittsburgh, Pennsylvania 15205. We are filing this Health and Safety Study to comply with the regulations codified at 40 CFR, Part 716. This submission contains no Confidential Business Information (CBI).

The information required at 40 CFR 716.30 is given below.

Chemical Name:	Toluene Diisocyanate; 2,4- and 2,6- (Desmodur T 80)
CAS No:	26471-62-5
Name of Study:	Salmonella/Microsome Test, Study # T 5039166 - Report 22479
Submitting Official:	Francis J. Rattay
Title:	Manager, Regulatory Affairs
Address:	Mobay Road Pittsburgh, Pa 15205
Telephone No.:	(412) 777-7471

Sincerely,

Francis J. Rattay /VMK

Francis J. Rattay
Manager, Regulatory Affairs
(412) 777-7471

Attachment
Certified Mail No.: P 921 654 216

B A Y E R A G
FACHBEREICH TOXICOLOGY
Friedrich-Ebert-Straße 217-333
D-5600 Wuppertal 1, F.R.G.

Report No. : 22479
Report Date: 31.08.1993

Desmodur T 80

SALMONELLA/MICROSOME TEST

Study No.: T 5039166

by

Dr. R. Gahlmann

This page is intentionally left blank for the purpose of submitting administrative information that is required by regulations promulgated by various countries.

GLP Certification by Study Director

Compound : Desmodur T 80
Study No. : T 5039166

This study conforms to OECD Principles of Good Laboratory Practice (Bundesanzeiger Nr. 42a of the 2nd of March 1983 and Bundesgesetzblatt, Part I, of the 22nd of March 1990).

Dr. R. Gahlmann

Wuppertal, December 22, 1992

CONTENTS

Declaration by Quality Assurance Unit

Compound : Desmodur T 80
Study No. : T 5039166

The laboratory in which this study was performed has been inspected by Quality Assurance on the dates indicated below. The results of the checks and inspections are conveyed in writing to the study director and, if necessary, also to the Head and Director of the Institute, or other responsible persons.

Date of check/inspection	Date of issue of inspection report
Aug. 13, 1992 (study plan)	Aug. 13, 1992
Oct. 22, 1992	Oct. 22, 1992

The results of this study and the methods used have been correctly reported.

Quality Assurance Unit
PH-AQ-S/GLP, Bayer AG

Date: August 27, 1993 Responsible: Lehn
Dr. H. Lehn

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

1. Signatures

Study Director : Dr. R. Gahlmann — AUG 30 1993 (Date)

Section Head : Dr. B. Herbold — AUG 30, 1993 (Date)

Head of Institute: Dr. E. Löser — Aug. 30.93 (Date)

2. Summary

The mutagenic potential of Desmodur T 80 was examined in the Salmonella/microsome test. Bacteria of four histidine-auxotrophic Salmonella typhimurium LT2 mutant strains (TA 98, TA 100, TA 1535, and TA 1537) were exposed to doses up to 5000 µg per plate.

All doses between 8 µg and 5000 µg per plate revealed bacteriotoxic effects: Total bacteria counts were reduced and/or growth inhibition was observed. The effects varied between strains and between the first and the repeat experiment and doses up to 5000 µg per plate could still be used for assessment purposes. Substance precipitation occurred at the dose of 200 µg per plate and above which impaired the assessment of some of the plates.

There was evidence for mutagenic effects of Desmodur T 80 with S9 mix. A biologically relevant and reproducible increase of the mutant count over control levels was observed at the doses between 125 and 1000 µg per plate with Salmonella typhimurium strain TA 98 and at the dose of 1000 µg per plate with strain TA 1537. Therefore, Desmodur T 80 was considered to be mutagenic with S9 mix in the Salmonella/microsome test. A positive response was found only with S9 mix. The lowest reproducible effective dose was 200 µg per plate for Salmonella typhimurium TA 98 and 1000 µg per plate for TA 1537. The Salmonella/microsome test thus showed Desmodur T 80 to have a weak but definite mutagenic effect.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene revealed marked mutagenic effects, as indicated by a biologically relevant increase of mutant colony numbers over colony numbers of the negative controls.

3. Introduction

The mutagenicity evaluation was performed by using the Salmonella/microsome test (Ames Test) as described by Ames et al. (1972a, 1975) and Maron and Ames (1983).

The Salmonella/microsome test is a screening method which allows to assess whether point mutations are induced by chemicals in the genome of Salmonella typhimurium test strains in vitro. Bacteria of auxotrophic mutant strains are exposed to the chemical agent and the number of revertants to a prototrophic phenotype is compared to the number of spontaneous revertants. A test agent is considered to be mutagenic if the rate of reversion increases significantly and reproducibly after treatment.

The mammalian metabolism which is an important factor in chemical mutagenesis is simulated in this test by the 9000 g fraction of homogenized mammalian livers. S9 mix which is composed of this liver cell extract, supplemented with cofactors, is added to the test system in order to mimic the metabolic features of mammalian cells.

The method itself is considered to be very sensitive (Herbold et al., 1976; Herbold, 1978) and is well suited for fast screening. Available literature indicates a high correlation between the positive and negative responses of the Ames assay and the carcinogenic activity of the tested substances (McCann et al., 1975a, 1976; Purchase et al., 1976, 1978). In addition, the test represents a good screening system for potential carcinogenic effects, although the results should not be overrated, as this high correlation may not apply to all substance groups (Ames, 1979; Andrews et al., 1978; Clayson, 1980; Glatt et al., 1979 and Rinkus and Legator, 1979; Zeiger, 1987).

The test was performed at the Institute of Toxicology for Industrial Chemicals, Fachbereich Toxicology, BAYER AG, Friedrich-Ebert-Straße 217-333, D-5600 Wuppertal 1, F.R.G.

Study initiation date:	August 10, 1992
Study start date:	October 20, 1992
Study termination date:	October 29, 1992
Study completion date:	report date (see front page)

The records are filed in the Fachbereich's archive.

4. Material and Methods

4.1 Substances

4.1.1 Test Substance

name of test substance	:	Desmodur T 80
order number	:	BALK 89/122
manufacturer	:	BAYER AG
product number	:	409 448
batch number	:	665
sample number	:	487 991
content	:	80.3 % (2,4-Toluenediisocyanate) 19.6 % (2,5- and 2,6-Toluenediisocyanate)
approved	:	until August 27, 1993
appearance	:	colourless liquid
storage	:	at room temperature
chemical name	:	2,4- and 2,6-Toluenediisocyanate (isomer mixture)
molecular weight	:	174.2 g/mole
molecular formula	:	$C_9H_6N_2O_2$
CAS No.	:	26471-62-5
intended use	:	industrial chemical

The batch used was analysed prior to study initiation and approved for use during the test period. A stability test did not reveal any significant change of the concentration of the active ingredient in the solvent over the test period.

4.1.2 Positive Controls

Sodium azide (Na-azid, SERVA), order no. 30175 (Control:D), a direct-acting mutagen used as specific positive control for TA 1535.

Nitrofurantoin (NF, SERVA), order no. 30600 (Control:A), a direct-acting mutagen used as specific positive control for TA 100.

4-nitro-1,2-phenylene diamine (4-NPDA, Merck), batch no. VV452057, a direct-acting mutagen used as specific positive control for TA 1537 and TA 98.

2-aminoanthracene (2-AA, EGA-Chemie), batch no. 7413406, a promutagen which reverts all the strains and serves as a control for the activating effect of the S9 mix.

The positive controls sodium azide, nitrofurantoin and 4-nitro-1,2-phenylene diamine were only used without S9 mix; the positive control 2-aminoanthracene was only used with S9 mix.

4.2 Indicator Organisms

4.2.1 Description of Test Strains

Histidine-deficient mutant strains of Salmonella typhimurium LT2 served as indicators of point mutagenic effects. The strains were selected specifically for the Salmonella/microsome test. Point mutations can be divided into two basic classes, base-pair substitutions and frameshift mutations. Thus, strains that allow to assay for both classes of mutations were included in the collection of test strains.

They included the Salmonella typhimurium strains TA 1535 and TA 1537 selected by Ames et al. (1973b) and TA 100 and TA 98 developed by McCann et al. (1975b). Both strain TA 1535 and TA 100 bear the base-pair substitution his G 46. TA 100 carries the plasmid pKM 101 which encodes the muc$^+$ gene that increases the resistance to the lethal effects of many mutagens at the expense of increased mutability and an ampicillin resistance gene as a selectable marker. The same R factor is also present in strain TA 98. TA 1537 and TA 98 bear the frameshift markers his C 3076 (a +1 mutant) and his D 3052 (a +2 mutant), respectively.

All strains have two additional properties in common which increase their sensitivity. Firstly, they are deep rough since certain lipopolysaccharide side chains are missing in the bacterial cell wall. Larger, mutagenic molecules can therefore enter the cell and cause mutations. Secondly, their reduced ability to repair damage from UV light (e.g. thymidine dimers) allows the phenotypic detection of mutation events which would otherwise remain undetected.

Strain TA 1535 is commonly used in addition to strain TA 100, while TA 1538 is normally not used in addition to TA 98. This has two reasons:

A) There is no relevant increase in the spontaneous mutant counts of TA 98, compared to the spontaneous range of TA 1538. Special differences in sensitivity existing between TA 1535 and TA 100, which are attributed to the relatively high spontaneous rate of TA 100 (10 times that of TA 1535), do not exist between TA 1538 and TA 98. b) An international general inquiry has shown, that using TA 1538 in addition to any of the test strains in this study would not provide further information of biological relevance (Herbold, 1983).

This is in agreement with international guidelines, as published by the OECD, EEC, or EPA. Strain TA 1538 was either deleted in these guidelines, or never introduced at all. Maron and Ames (1983) also reported: "Although TA 1538 is useful for the detection of particular aromatic frameshift mutagens such as 4-nitro-o-phenylene diamine, we decided to drop the strain because it overlaps considerably with TA 98.

4.2.2 Origin of Strains

The original strains were obtained from Prof. Bruce Ames and arrived at the Fachbereich Toxicology, BAYER AG, on December 12, 1986.

4.2.3 Production of Stock Cultures

The samples were inoculated immediately upon receipt onto nutrient agar plates and incubated at 37°C for approximately 24 hours. Plates and medium that were used for the cultivation of strain TA 100 and TA 98 at this step and during following selection procedures contained ampicillin. Nutrient broth was inoculated with single colonies and cultures for each strain were grown over night at 37°C. Bacteria from each culture were then grown on nutrient agar plates.

After an incubation period of approximately 24 hours at 37°C, new samples of individual colonies from these plates were transferred to flasks containing approximately 30 ml of standard nutrient broth. The culture was incubated overnight at 37°C. Thereafter, a small sample was removed to check the genotype. The remaining cultures were treated with DMSO to protect against the effects of freezing, and immediately frozen in portions of 1 ml at -80°C (Ames et al., 1973b; McMann et al., 1975b). No additional ampicillin-resistance tests were required for strains TA 98 and TA 100 since the bacteria had already been grown under ampicillin selection.

The crystal-violet sensitivity test (to confirm the deep rough phenotype) and UV sensitivity test (to confirm the phenotype) were performed as described below. Frozen cultures which did not meet the criteria were discarded. Remaining cultures were stored for future testing. Frozen cultures of batches that produced results deviating from expected values for negative or positive controls during mutagenicity testing were also discarded.

Whenever new stock cultures were needed, cultures were inoculated from single colonies from stock plates which contained ampicillin in the nutrient agar for the strains TA 100 and TA 98. The cultures containing approximately 30 ml of nutrient broth were incubated, stored in aliquots of 1 ml, and checked for crystal-violet and UV sensitivity.

One 1 ml-portion was thawed for each test and strain, and quantities of 0.2 ml of the thawed culture were added to 10 ml nutrient broth. This culture was incubated overnight at 37°C and used only on the same day. Thus, each test was performed with bacteria that had been grown from aliquots of a small stock culture whose properties had been checked immediately before freezing. In general, this obviated any need to re-check the genotype for each Salmonella/microsome test. This procedure is in accordance with the methods described by Ames et al. (1975) and Maron and Ames (1983).

4.2.4 Checking of Genotype

4.2.4.1 Histidine Requirement

In each individual test, histidine dependence of the cultures was automatically checked by the accompanying negative controls. The number of mutants of each individual plate is listed in the Tables 1 to 8.

4.2.4.2 Ampicillin Resistance (pKM 101)

A special test for ampicillin resistance was not necessary since strains TA 100 and TA 98 were incubated on ampicillin containing nutrient agar. Consequently surviving bacteria were ampicillin resistant.

4.2.4.3 Crystal-Violet Sensitivity (deep rough)

A volume of 0.1 ml was taken from individual stock samples and spread on nutrient agar plates (four plates per strain). After a few minutes, filter papers to which 10 µl of an aqueous crystal-violet solution (1 mg/ml) had been added were placed in the middle of the plates. The plates were incubated overnight at 37°C and the diameters of the inhibition zones that had formed were measured. The inhibition zones of all batches of stocks that were used for mutagenicity testing revealed adequate sensitivity to crystal-violet.

4.2.4.4 UV Sensitivity (uvrB)

Samples were spread onto nutrient agar plates as described under 4.2.4.3. One half of each plate was covered with aluminium foil and irradiated with UV light of a wavelength of 254 nm at a distance of 33 cm without a lid for six seconds (TA 1535 and TA 1537) or eight seconds (TA 100 and TA 98), respectively. The irradiated plates were incubated as described under 4.2.4.3 and inspected. Adequate sensitivity was demonstrated if no bacteria had grown on the irradiated half of the plate. This was the case with all batches of stocks that were used for mutagenicity testing.

4.2.5 Stock Batches

Stock Batches Used in Tables 1-4	5-8	Strain
24.07.92/1	24.07.92/1	TA 1535
24.07.92/1	24.07.92/1	TA 100
24.07.92/1	24.07.92/1	TA 1537
24.07.92/1	17.09.92/1	TA 98

4.3 S9 Mix

S9 mix was used to simulate the mammalian metabolism of the test substance. It was prepared from the livers of at least six adult male Sprague Dawley rats of approximately 200 to 300 g in weight. For enzyme induction, the animals received a single intraperitoneal injection of Aroclor 1254, dissolved in corn oil, at a dose of 500 mg/kg body weight, five days before sacrifice. The animals were prepared unfasted, following the directions of Ames et al.(1975) and Maron and Ames (1983).

The rats were killed by cervical dislocation. Livers were removed under sterile conditions immediately after sacrifice and kept at 4°C until all animals had been prepared. All the remaining steps were carried out under sterile conditions at 4°C.

The livers were washed with a cold (+4°C) solution of 0.15 M KCl (approximately 1 ml KCl per gram of liver) and homogenized in fresh, cold (+4°C) 0.15 M KCl (approximately 3 ml KCl per gram of liver). The homogenate was then centrifuged in a precooled centrifuge at +4°C and 9000 g for 10 minutes. The supernatant (the S9 fraction) was stored at -80°C in small portions.

Aliquots of the frozen supernatant were thawed slowly before use. The S9 mix was prepared freshly each time (Ames et al., 1973a) and used only on the same day. Throughout the experiment, the mix was kept cold in a glass vessel with a double wall in which the space between the walls had been filled with ice water.

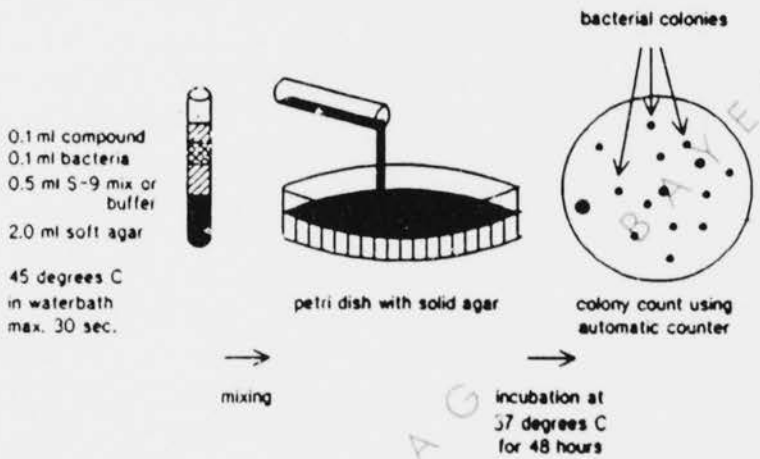
Seventy ml of cofactor solution contained:

$MgCl_2 \times 6\ H_2O$	162.6 mg
KCl	246.0 mg
glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
phosphate buffer	100.0 mM

S9 mix consists of this cofactor solution, S9 fraction and, if needed, 0.15 M KCl. The amount of S9 fraction in S9 mix is indicated in Tables 1 to 8 in percent. The S9 mix comprised the amount of S9 fraction (x%) indicated in Tables 1 to 8, 70% cofactor solution and (30-x)% 0.15 M KCl. The S9 fractions were derived from the preparation dated July 27, 1992 (protein content: 27.2 mg per ml per ml). Prior to first use, each batch was checked for its metabolizing capacity by using reference mutagen(s) and appropriate activity was demonstrated. At the beginning of each experiment 4 aliquots of the S9 mix were plated (0.5 ml/plate) in order to assess its sterility. This was repeated after finishing of test tube plating. The sterility control plates were then incubated for 48 hours at 37°C. No indication of contamination of S9 mix was found.

4.4 Test Protocol

The test followed the directions of Ames et al. (1973a, 1975) and Maron and Ames (1983).



Four tubes were plated per strain and dose for the mutant count, with and without S9 mix, respectively. As negative controls the same number of tubes with solvent minus the test substance was plated. Positive controls were also plated in quadruplicate. The amount of solvent that was used for the test substance and for the controls was 0.1 ml/plate.

In general, tubes were plated immediately after addition of the last component. In some cases, however, a preincubation of the test tubes was performed before plating. This was not the case in the present study.

The doses for the first trial were normally determined on the basis of a standard protocol: 5000 µg or 5 µl per plate were used as the highest dose unless the solubility was limiting. At least four additional doses were routinely used. If plates of fewer than three doses could be used for assessment purposes, at least two repeat tests were performed. The results of the first experiment were then regarded as a pre-test for toxicity. In case of a positive response, however, or if the plates of at least three doses could be used for assessment purposes, the first trial was included in the assessment. If the second test confirmed the results of the first test, no additional repeat test was performed. Doses of repeat tests were chosen on the basis of the results obtained in the first experiment.

The toxicity of the substance was assessed in three ways. First, background growth on the plates for mutant determination was inspected. If a reduction in background growth was observed, this indication for toxicity was indicated in the tables by the letter "B" after the mutant count. A single "B" without any numerical value for a mutant count represents four plates with reduced background growth at a given concentration. (The same applies to the symbols "C", "V", "P", "N" or "%", which may also appear in the tables.) Secondly, a toxic effect of the substance was assumed when the mutant count per plate was reduced significantly and in a dose-dependent manner as compared to the corresponding negative control. The third criterion was the bacteria titer. Total bacterial counts were taken on two plates with S9 mix for each concentration studied. If a test was performed only without S9 mix, however, the bacterial count was taken on plates without S9 mix.

The bacterial suspensions were obtained from 17-hour cultures in nutrient broth, which had been shaken at 37°C and at 90 rpm. Such suspension cultures were used for the plating experiments. No standardized procedure was employed to adjust the bacterial suspensions to a defined density of viable cells per milliliter, since the selected culture conditions normally produce cultures of the desired density. However, the numbers of viable cells in each culture were determined as part of the titration procedure. The numbers of viable cells are listed in Tables 1 to 8 as the negative control values.

The dilution of bacterial suspensions used for the determination of titers was 1:1,000,000. Plating for the titration and for mutagenicity testing was performed under the same conditions except that the histidine concentration in the soft agar was raised from 0.5 mM to 2.5 mM for the titration to permit unrestricted bacteria growth.

The tests were generally performed with and without S9 mix. Details of the results are compiled in Tables 1 to 8.

The plates were incubated at 37°C for 48 hours and bacteria colonies were generally counted immediately after incubation. If no immediate count was possible, plates were temporarily stored in a refrigerator.

The following criteria determined the acceptance of an assay:
a) The negative controls had to be within the expected range, as defined by published data (i.e. Maron and Ames, 1983) and our historical data (see Chapter 8).
b) The positive controls had to show sufficient effects, as defined by the laboratories' experience (see Chapter 8).
c) Titer determinations had to demonstrate sufficient bacterial density in the suspension.

Only assays which complied with all three of the above criteria were used for assessment. Furthermore, the data generated in this assay needed to be confirmed by two additional independent experiments. Even if the criteria for points (a), (b) and (c) were not met, an assay was accepted if it showed mutagenic activity of the test compound.

The following doses per plate were evaluated in the first test:

		µg per plate	
1.	Negative control	0	
2.	Desmodur T 80	5000	
3.	Desmodur T 80	1000	
4.	Desmodur T 80	200	
5.	Desmodur T 80	40	
6.	Desmodur T 80	8	
7.	Positive control, sodium azide	10	(only TA 1535)
8.	Positive control, nitrofurantoin	0.2	(only TA 100)
9.	Positive control, 4-nitro-1,2-phenylene diamine	10	(only TA 1537)
10.	Positive control, 4-nitro-1,2-phenylene diamine	0.5	(only TA 98)
11.	Positive control, 2-aminoanthracene	3	

Due to the substance's toxicity and precipitation, doses ranging from 125 µg to 4000 µg per plate were chosen for the repeat tests. Individual doses are given in Tables 5 to 8.

The solvent employed for Desmodur T 80 was ethylene glycol dimethylether (EGDE) and for the positive controls DMSO.

The solvent for the test substance was selected from a priority list in the order water, methanol, ethanol, acetone, DMSO, DMF, and ethylene glycol dimethylether (EGDE) according to the information provided by the internal sponsor that the test substance might not be stable in alternative solvents including DMSO.

No "untreated" negative control was set up for EGDE, since sufficient evidence was available in the literature (i.e. Maron and Ames, 1983) and from our own experience (see Chapter 8), indicating that this solvent had no influence on the numbers of spontaneous revertants with the bacterial strains used.

4.5 Assessment of Results

A test is defined as being positive if a reproducible and dose-related increase of mutant colony numbers becomes apparent for at least one strain. For TA 1535, TA 100 and TA 98 mutant colony numbers should increase by a factor of two or more over negative control numbers, while at least a three-fold increase should be apparent for TA 1537. Otherwise, the result is judged as negative. However, these guidelines may be overruled by good scientific judgement.

In case of questionable results, investigations should continue, possibly with modifications, until a final evaluation is possible.

4.6 Study Guidelines

The study was performed according at least to the following guidelines:

EEC Directive 84/449/EEC
B.14. Other Effects - Mutagenicity
Salmonella typhimurium
Reverse Mutation Test

OECD Guidelines for Testing of Chemicals
"Genetic Toxicology: Salmonella typhimurium, Reverse Mutation Assay"
Adopted: 26 May 83, No. 471

New and Revised Health Effects Test Guidelines October 1984. (U.S.) Environmental Protection Agency Washington, DC (PB 84-233295).
HG - Gene Muta - S. typhimurium, October 1984

4.7 Study Identification and Responsibilities

4.7.1 Type of Test and Study Number

Salmonella/Microsome Test :T 5039166

4.7.2 Responsibilities

Head of Institute of Toxicology for Industrial Chemicals	:Dr. E. Löser
Section Head	:Dr. B. Herbold
Study Director	:Dr. R. Gahlmann
Senior Technician	:Mrs. M. Bönning
Head of Archives	:Dr. E. Löbbecke
Quality Assurance	:Dr. H. Lehn
Analysts	:Dr. Gießler/Mr. Kulinna

5. Results

5.1 Description of Results

The mean values of the mutant counts for each set of plates are listed in Tables 1 to 8. There was indication of bacteriotoxic effects of Desmodur T 80 at all doses between 8 and 5000 µg per plate. The total bacteria counts were reduced as compared to numbers for the negative controls or growth inhibition was observed. The effects differed between strains and between the first and the repeat experiment. Nevertheless, doses up to 5000 µg per plate could still be used for assessment purposes.

The substance started to precipitate at a dose of 200 µg per plate.

Two of the four test strains revealed a dose-related and reproducible increase of revertant colony numbers by a factor of two to four as compared to the numbers of the corresponding negative control plates. Strains TA 98 and TA 1537 were affected (Tables 3,4 and 7,8).

The lowest dose at which this finding was reproducible was approximately 200 µg per plate for Salmonella typhimurium TA 98 (Tables 4 and 8), and approximately 1000 µg per plate for Salmonella typhimurium TA 1537 (Tables 3 and 7). Positive findings were obtained only with S9 mix.

The increase for Salmonella typhimurium TA 100 with S9 mix was only observed in the repeat test (Table 6).

Summary of the Results with
Desmodur T 80
in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98
without	-ve	-ve	-ve	-ve
with	-ve	-ve	+ve	+ve

-ve = negative, +ve = positive

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene raised mutant counts well over negative control levels. This demonstrated the system's sensitivity and the activity of the S9 mix.

5.2 Tabulated Summary of Data

Summary of Mean Values without S9 Mix from Tables 1-8

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
1-4				
0	9	42	8	21
8	10	70	5	21
40	7	28	4	16
200	1	28	2	10
1000	0	6	/	0
5000	0	2	/	/
Na-azide	567			
NF		176		
4-NPDA			71	213
5-8				
0	12	55	9	25
125	14	42	6	18
250	9	43	7	13
500	6	32	3	5
1000	4	15	1	2
2000	0	2	/	0
4000	0	0	/	/
Na-azide	590			
NF		256		
4-NPDA			57	84

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of Mean Values with S9 Mix from Tables 1-8

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
1-4 10% S9				
0	12	73	7	29
8	8	87	12	35
40	13	88	7	56
200	9	85	16	110
1000	7	94	31	122
5000	4	/	/	/
2-AA	163	900	98	1045
5-8 10% S9				
0	14	71	9	36
125	1.	143	12	86
250	12	188	15	101
500	12	211	21	123
1000	4	57	28	88
2000	2	21	2	23
4000	/	/	/	/
2-AA	92	869	306	888

6. Assessment

All doses of Desmodur T 80 between 8 and 5000 µg per plate did induce bacteriotoxic effects in the Salmonella/microsome test: Total bacteria counts were changed and/or growth inhibition was observed. Doses up to 5000 µg per plate could still be used for assessment purposes.

Substance precipitation occurred at 200 µg per plate and above.

Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility), revealed clear, biologically relevant variations from the respective negative controls for TA 98 and TA 1537. These were regarded as mutagenic effects of Desmodur T 80. Since the lowest effective doses at which these findings were reproducible, was in the medium and bacteriotoxic dose range, Desmodur T 80 is considered to be a weak but definite mutagen.

In spite of the low doses used, positive controls increased the mutant counts significantly over negative control levels which demonstrated the system's high sensitivity.

Due to this sensitivity, clear indication of mutagenic effects of Desmodur T 80 could be found at assessable doses up to 1000 µg per plate in Salmonella typhimurium strains TA 98 and TA 1537.

7. References

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8. Historical Controls

Summary of historical negative and positive controls of experiments performed from January to June 1988 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	14	2	97	9	8	1	17	2
DMSO	-	13	2	94	15	8	1	17	2
DMF	-	12	2	87	11	8	1	19	3
ethanol	-	15	3	69	7	7	1	22	3
acetone	-	10	2	85	10	7	1	18	2
EGDE[2]	-	18		117		10		21	
Na-azide	-	839	115						
NF	-			382	46				
4-NPDA	-					90	13	109	20
30%									
water	+	14	3	134	10	8	2	29	3
DMSO	+	15	3	124	14	9	2	29	3
DMF	+	14	3	113	9	9	2	31	5
ethanol	+	20	2	105	6	6	1	30	3
acetone	+	14	1	134	25	11	2	34	3
EGDE[2]	+	18		159		9		35	
2-AA	+	282	63	601	164	66	17	532	160
10%									
water	+	14	4	123	4	9		33	
DMSO	+	14	2	111	13	8	1	33	5
DMF	+	--		72		9		27	
ethanol	+	23		87	6	8		38	
acetone	+	13		85		7		29	
2-AA	+	357	67	1422	428	298	65	1323	323

[2]) Ethylene glycol dimethylether

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of historical negative and positive controls of experiments performed from July to December 1988 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water	-	14	3	97	9	8	2	20	5
DMSO	-	14	2	93	25	8	1	19	10
DMF	-	12	2	70	4	7	1	13	1
ethanol	-	10	2	71	2	7	1	21	3
acetone	-	15	2	138	10	8	3	39	6
Na-azide	-	822	137						
NF	-			412	42				
4-NPDA	-					88	19	124	25
30%									
water	+	12	2	144	15	10	2	35	6
DMSO	+	16	2	124	15	10	2	32	5
DMF	+	14	3	117	14	9	2	31	6
ethanol	+	17	3	90	4	8	1	39	2
acetone	+	13	4	177	35	9	2	43	8
2-AA	+	261	69	755	196	93	21	583	171
10%									
DMSO	+	7	1	110	12	9	1	32	5
DMF	+	11		121		6		26	
2-AA	+	148	70	1544	572	416	75	1499	423

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of historical negative and positive controls of experiments performed from January to June 1989 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	10	3	91	11	7	1	18	3
DMSO	-	9	3	84	16	7	2	16	2
DMF	-	7	1	60	4	6	1	14	2
ethanol	-	10	2	73	12	7	2	18	4
acetone	-	9	-	100	--	7	-	18	-
EGDE[2]	-	8	2	69	16	6	2	17	4
Na-azide	-	721	110						
NF	-			359	61				
4-NPDA	-					75	13	119	35
30%									
water	+	14	2	133	12	9	2	32	8
DMSO	+	14	3	114	18	9	1	28	4
DMF	+	14	2	100	9	8	2	25	4
ethanol	+	17	3	118	12	10	2	37	8
acetone	+	15	-	138	--	13	-	32	-
EGDE[2]	+	14	2	115	25	11	2	27	8
2-AA	+	195	33	633	127	63	28	392	133
10%									
DMSO	+	12	2	105	28	7	2	25	4
DMF	+	--	-	---	--	7	-	31	-
2-AA	+	267	27	1455	348	283	64	1547	289

[2]) Ethylene glycol dimethylether

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of historical negative and positive controls of experiments performed from July to December 1989 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
DMSO	-	10	4	72	6	7	4	16	5
DMF	-	9	4	57	15	8	2	17	5
ethanol	-	8	3	57	12	6	1	14	6
acetone	-	15	-	96	--	6	-	13	-
EGDE[2]	-	8	-	63	--	6	-	21	-
Na-azide	-	853	147						
NF	-			326	47				
4-NPDA	-					91	26	87	25
30%									
DMSO	+	14	2	89	7	11	2	23	2
DMF	+	15	3	87	6	11	4	26	4
ethanol	+	11	6	79	13	8	2	23	5
acetone	+	21	-	96	--	11	-	20	-
EGDE[2]	+	13	-	87	--	11	-	26	-
2-AA	+	157	42	500	83	73	22	498	101
10%									
DMSO	+	14	5	91	7	10	1	24	4
ethanol	+	11	-	53	--	4	-	18	-
2-AA	+	158	54	1464	152	289	117	1294	113

[2]) Ethylene glycol dimethylether

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of historical negative and positive controls of experiments performed from January to June 1990 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water -	15	3	74	10	7	1	22	5
DMSO -	12	2	72	13	8	2	17	3
DMF -	10	4	65	10	7	2	10	6
methanol-	17		87		7		19	
ethanol -	13	3	77	11	8	2	19	2
acetone -	10	1	69	4	6	1	11	2
EGDE[2] -	14	4	95	14	8	1	18	5
Na-azide-	799	108						
NF -			268	48				
4-NPDA -					52	12	81	14
30%								
water +	18	2	108	17	9	2	27	5
DMSO +	18	3	86	11	9	2	27	3
DMF +	13	3	97	17	7	3	20	5
methanol+	22		121		11		28	
ethanol +	19	3	98	15	8	2	29	4
acetone +	13	1	104	8	7	3	22	3
EGDE[2] +	15	2	97	9	9	3	28	8
2-AA +	161	39	509	130	48	15	379	54
10%								
DMSO +	18	2	89	20	11	4	30	6
ethanol +	16		85		8		29	
acetone +			107				17	
2-AA +	214	49	1196	181	235	38	1140	284

[2]) Ethylene glycol dimethylether

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of historical negative and positive controls of experiments performed from July to December 1990 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	13	2	105	16	9	1	21	4
DMSO	-	14	2	105	7	8	1	21	3
DMF	-	13	2	82	16	6	2	12	4
methanol	-	13	1	105	16	8	1	21	4
ethanol	-	12	3	93	14	9	1	22	3
acetone	-	12	2	116	2	6	1	23	1
EGDE[2]	-	13	2	112	15	8	2	18	3
Na-azide	-	882	114						
NF	-			380	60				
4-NPDA	-					48	9	71	15
30%									
water	+	18	3	143	15	11	2	29	3
DMSO	+	17	2	137	5	10	2	28	4
DMF	+	15	3	109	14	10	1	23	3
methanol	+	22	2	144	16	11	2	33	3
ethanol	+	19	3	118	18	10	1	39	7
acetone	+	13	1	131	4	9	1	26	1
EGDE[2]	+	18	3	135	14	11	2	32	5
2-AA	+	175	41	800	243	84	17	185	93
10%									
DMSO	+	16	2	127	19	9	3	32	5
acetone	+	12		124		10		26	
EGDE[2]	+			140					
2-AA	+	179	69	1321	148	298	39	1206	168

[2]) Ethylene glycol dimethylether

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of historical negative and positive controls of experiments performed from January to June 1991 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water -	12	3	111	10	9	2	28	5
DMSO -	13	2	113	14	10	2	30	3
DMF -	9	-	80	--	7	-	23	-
methanol-	11	2	105	14	8	2	29	5
ethanol -	12	1	96	15	9	2	31	5
acetone -	10	-	55	--	5	-	21	-
EGDE[2] -	11	3	108	5	8	1	23	8
Na-azide-	623	102						
NF -			398	56				
4-NPDA -					49	10	89	20
30%								
water +	16	3	152	15	12	2	38	7
DMSO +	18	3	154	11	12	2	40	7
DMF +	11	-	84	--	9	-	29	-
methanol+	23	5	152	7	10	3	48	10
ethanol +	19	3	127	17	10	3	43	6
acetone +	14	-	84	--	14	-	18	-
EGDE[2] +	15	4	132	6	8	1	40	9
2-AA +	182	33	800	163	86	24	472	105
10%								
water +	15	-	102	--	5	-	46	-
DMSO +	16	3	132	5	10	1	39	4
methanol+	--	-	150	--	--	-	--	-
2-AA +	208	48	1408	216	314	14	754	369

[2]) Ethylene glycol dimethylether

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
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Summary of historical negative and positive controls of experiments performed from July to December 1991 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water -	12	3	89	10	9	3	27	4
buffer -	13	2	97	10	8	1	25	2
DMSO -	12	3	92	15	9	1	24	4
DMF -	7		75		7		17	
methanol-	10	1	84	11	8	1	25	3
ethanol -	12	4	80	8	8	3	23	4
acetone -	12	2	87	6	8	1	26	4
EGDE[2] -	14	3	107	22	8	1	26	5
Na-azide-	605	122						
NF -			339	52				
4-NPDA -					53	9	79	17
30%								
water +	19	4	138	21	13	2	33	4
buffer +	17		159		13		38	
DMSO +	19	3	130	11	10	2	33	4
DMF +	11		142		9		32	
methanol+	25		134		12		37	
ethanol +	18	5	119	19	11	2	37	2
acetone +	18	2	111	9	13		28	11
EGDE[2] +	22	4	144	11	13	3	32	3
2-AA +	164	38	727	139	91	32	520	161
10%								
water +	16	4	113	18	10	3	33	5
buffer +	14		94		10		34	
DMSO +	16	2	118	14	10	3	31	3
DMF +	15		114	6	11		21	
methanol+	16		111		9		29	
ethanol +	19	3	94	6	12	2	32	2
acetone +	17		112		11		32	
EGDE[2] +	20	2	153	11	11	1	34	5
2-AA +	197	50	1431	260	304	116	1097	207

[2]) Ethylene glycol dimethylether

Desmodur T 80
Salmonella/Microsome Test
Study No. T 5039166
BAYER AG

Summary of historical negative and positive controls of experiments performed from January to June 1992 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water	-	16	2	93	11	9	2	23	4
DMSO	-	15	4	81	9	9	1	23	4
DMF	-	13	3	68	4	7	1	19	3
ethanol	-	15	4	91		7	2	25	2
acetone	-	13	2	59	12	7	1	19	1
EGDE[2]	-	17		69		7		14	
Na-azid	-	660	147						
NF	-			285	65				
4-NPDA	-					52	7	74	13
30%									
water	+	23	4	124	18	12	1	31	6
DMSO	+	22	5	120	20	12	2	31	5
DMF	+	18	2	89	5	11	1	27	1
ethanol	+	25		145		9		38	
acetone	+	17	3	79	9	8	2	24	4
EGDE[2]	+	19		119		8		18	
2-AA	+	151	17	669	208	62	13	382	111
10%									
water	+	20	4	118	17	12	3	33	5
DMSO	+	17	3	111	15	10	2	32	4
DMF	+	17		87		9		34	
ethanol	+	24	4	98	5	9	2	37	5
acetone	+	15	3	69	7	12	6	29	5
EGDE[2]	+	11		48		11		22	
2-AA	+	159	35	1148	332	246	21	1126	292

[2]) Ethylene glycol dimethylether

9. Stability in Vehicle

Results of the analysis for stability of
Desmodur T 80
in the vehicle at room temperature

nominal value in mg/ml	content in % after storage time 0 hrs	4 hrs
0.02	94.6	95.7
50	99.4	96.7

According to these results Desmodur T 80 is stable in the vehicle at room temperature at concentrations ranging from 0.02 mg/ml to 50 mg/ml for at least four hours.

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 80

Table : 1

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 22, 1992
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	9 13 7 5	9	3	16 16 4 11	12	6	27 18	2.3	1.0	1.0
8	12 11 6 9	10	3	15 4 3 11	8	6	44 60	5.2	1.1	0.7
40	10 4 5 9	7	3	17 B 10 B 10 B 13 B	13	3	79 8	4.4	0.8	1.1
200	1 P 0 P 1 P 2 P	1	1	13 P 8 P 6 B 8 B	9	3	67 0	3.4	0.1	0.7
1000	0 P 0 P 0 P 0 P	0	0	8 P 7 P 7 P 6 P	7	1	0 P 0 P	< 0.1**	0.0	0.6
5000	0 P 0 P 0 P 0 P	0	0	6 P 2 P 6 P 0 P	4	3	0 P 0 P	< 0.1**	0.0	0.3
Na-azide 10	706 686 530 347	567	167	%	/	/	38 4	2.1	66.7*	/
2-AA 3	%	/	/	208 185 130 128	163	40	50 62	5.6	/	13.9*

*: Mutagenic effect
P: Precipitation
**: Bacteriotoxic effect
M: Mean
-S9: without S9 Mix

%: not tested
B: Background lawn reduced

SD: Standard-Deviation
+S9: with S9 Mix

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 80

Table : 2

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 22, 1992
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	29	42	11	62	73	7	17	1.9	1.0	1.0
	47			74			20			
	55			78						
	38			77						
8	70	70	3	72	87	21	4	0.4**	1.7	1.2
	74			117			4			
	68			82						
	68			75						
40	14 B	28	12	96 B	88	16	6	0.6**	0.7	1.2
	27 B			74 B			5			
	26 B			75 B						
	44 B			107 B						
200	31 B	28	11	90 B	85	32	0	< 0.1**	0.7	1.2
	15 B			63 B			0			
	25 B			60 B						
	41 B			128 B						
1000	20 P	6	10	114 B	94	22	3 P	0.4**	0.1	1.3
	3 P			94 B			4 P			
	0 B			64 P						
	0 B			105 P						
5000	3 P	2	3	P	/	/	P	/	0.1	/
	6 P									
	0 P									
	0 P									
NF 0.2	208	176	29	%	/	/	180	15.0	4.2*	/
	182						120			
	175									
	138									
2-AA 3	%	/	/	1047	900	143	283	20.8	/	12.4*
				990			132			
				823						
				739						

*: Mutagenic effect
P: Precipitation
**: Bacteriotoxic effect
M: Mean
-S9: without S9 Mix

%: not tested
B: Background lawn reduced

SD: Standard-Deviation
+S9: with S9 Mix

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 80

Table : 3

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 22, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	4 4 11 12	8	4	5 6 8 9	7	2	16 14	1.5	1.0	1.0
8	3 B 6 B 2 B 7 B	5	2	10 B 12 B 13 B 11 B	12	1	53 14	3.4	0.6	1.6
40	2 B 4 B 4 B 7 B	4	2	10 B 9 B 6 B 4 B	7	3	34 32	3.3	0.5	1.0
200	1 B 3 B 0 B 3 B	2	2	9 B 15 B 18 B 20 B	16	5	0 0	< 0.1**	0.2	2.2
1000	B B P P	/	/	33 P 35 P 26 P 30 P	31	4	P	/	/	4.4*
5000	P	/	/	P	/	/	P	/	/	/
4-NPDA 10	50 67 86 81	71	16	%	/	/	23 50	3.7	9.2*	/
2-AA 3	%	/	/	122 137 64 67	98	37	17 7	1.2	/	13.9*

*: Mutagenic effect
P: Precipitation
**: Bacteriotoxic effect
M: Mean
-S9: without S9 Mix

%: not tested
B: Background lawn reduced

SD: Standard-Deviation
+S9: with S9 Mix

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 80

Table : 4

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 22, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10⁻⁶	per ml 10⁻⁸	QUOTIENT -S9	+S9
EGDE	29	21	6	33	29	5	40	2.4	1.0	1.0
	23			25			7			
	19			25						
	14			33						
8	23	21	4	31	35	3	45	4.0	1.0	1.2
	23			38			35			
	23			33						
	16			37						
40	16	16	3	58	56	9	12	0.9	0.7	1.9
	20			42			5			
	14			61						
	13			61						
200	12 B	10	3	138	110	22	24	1.2	0.5	3.8*
	13 B			118			0			
	6 B			94						
	8 B			91						
1000	0 B	0	0	100 P	122	15	11 P	0.6**	0.0	4.2*
	0 B			129 P			0 P			
	0 B			134 P						
	0 B			126 P						
5000	P	/	/	P	/	/	P	/	/	/
4-NPDA 0.5	158	213	60	%	/	/	111	10.5	10.0*	/
	191						98			
	205									
	299									
2-AA 3	%	/	/	1269	1045	229	123	11.9	/	36.0*
				1190			115			
				956						
				766						

*: Mutagenic effect
P: Precipitation
**: Bacteriotoxic effect
M: Mean
-S9: without S9 Mix

%: not tested
B: Background lawn reduced

SD: Standard-Deviation
+S9: with S9 Mix

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 80

Table : 5

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 29, 1992
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10⁻⁶	TITER per ml 10⁻⁸	QUOTIENT -S9	QUOTIENT +S9
EGDE	15 14 11 9	12	3	16 12 18 9	14	4	203 241	22.2	1.0	1.0
125	15 8 18 15	14	4	13 7 16 15	13	4	230 186	20.8	1.1	0.9
250	10 12 5 7	9	3	11 12 12 11	12	1	200 199	20.0	0.7	0.8
500	10 P 5 P 3 P 6 P	6	3	15 P 12 P 10 P 9 P	12	3	188 161	17.5	0.5	0.8
1000	4 P 5 P 2 P 3 P	4	1	3 P 3 P 4 P 7 P	4	2	152 P 166 P	15.9	0.3	0.3
2000	0 B 0 B 0 P 0 P	0	0	3 P 2 P 4 B 0 B	2	2	41 P 28 P	3.5**	0.0	0.2
4000	0 B 0 B 0 P 0 P	0	0	P P B B	/	/	P P	/	0.0	/
Na-azide 10	576 530 564 688	590	68	%	/	/	191 189	19.0	48.1*	/
2-AA 3	%	/	/	87 96 95 89	92	4	191 191	19.1	/	6.7*

*: Mutagenic effect
P: Precipitation
**: Bacteriotoxic effect
M: Mean
-S9: without S9 Mix

%: not tested
B: Background lawn reduced

SD: Standard-Deviation
+S9: with S9 Mix

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 80

Table : 6

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 29, 1992
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	50 57 44 69	55	11	73 63 62 85	71	11	97 78	8.8	1.0	1.0
125	36 49 31 52	42	10	139 148 149 136	143	6	137 96	11.7	0.8	2.0
250	34 49 36 51	43	9	174 197 171 211	188	19	99 114	10.7	0.8	2.7
500	39 P 31 P 26 P 30 P	32	5	266 P 222 P 197 P 159 P	211	45	106 97	10.2	0.6	3.0*
1000	13 P 22 P 11 P 14 P	15	5	52 P 45 P 48 P 84 P	57	18	95 P 85 P	9.0	0.3	0.8
2000	4 B 0 B 2 P 0 P	2	2	44 B 8 B 29 P 4 P	21	19	48 P 50 P	4.9	0.0	0.3
4000	0 B 0 B 0 P 0 P	0	0	B B P P	/	/	P P	/	0.0	/
NF 0.2	303 241 231 249	256	32	%	/	/	131 124	12.8	4.7*	/
2-AA 3	%	/	/	999 878 652 945	869	153	122 155	13.9	/	12.3*

*: Mutagenic effect
P: Precipitation
**: Bacteriotoxic effect
M: Mean
-S9: without S9 Mix

%: not tested
B: Background lawn reduced

SD: Standard-Deviation
+S9: with S9 Mix

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : Desmodur T 80

Table : 7

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 29, 1992
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	9	9	2	5	9	3	68	6.9	1.0	1.0
	10			12			69			
	7			10						
	11			8						
125	3	6	4	16	12	3	97	11.0	0.6	1.3
	2			11			122			
	11			11						
	6			8						
250	7	7	2	17	15	2	122	12.5	0.7	1.7
	9			12			127			
	4			16						
	6			14						
500	3 P	3	1	19 P	21	2	114	11.0	0.4	2.4
	3 P			19 P			106			
	2 P			23 P						
	5 P			22 P						
1000	0 B	1	1	29 B	28	8	160 P	15.4	0.1	3.2*
	2 B			17 B			148 P			
	1 P			30 P						
	0 P			36 P						
2000	B	/	/	7 B	2	4	P	/	/	0.2
	B			0 B			P			
	P			0 P						
	P			0 P						
4000	B	/	/	B	/	/	P	/	/	/
	B			B			P			
	P			P						
	P			P						
4-NPDA 10	56	57	10	%	/	/	142	16.4	6.2*	/
	48						186			
	53									
	71									
2-AA 3	%	/	/	241	306	53	182	16.7	/	35.0*
				367			152			
				321						
				296						

*: Mutagenic effect
P: Precipitation
M: Mean
-S9: without S9 Mix
%: not tested
B: Background lawn reduced
SD: Standard-Deviation
+S9: with S9 Mix

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WUPPERTAL ELBERFELD
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Table : 8

Study Number : T 5039166
Study Director : Dr.Gahlmann
Technician : Meier
Date : Oct. 29, 1992
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
EGDE	28 27 27 19	25	4	37 37 31 40	36	4	125 114	12.0	1.0	1.0
125	17 19 12 24	18	5	82 101 91 70	86	13	119 151	13.5	0.7	2.4*
250	17 14 4 15	13	6	73 108 116 108	101	19	152 153	15.3	0.5	2.8*
500	5 P 4 P 6 P 6 P	5	1	99 P 99 P 145 P 147 P	123	27	137 P 149 P	14.3	0.2	3.4*
1000	3 B 2 B 0 P 4 P	2	2	75 P 51 P 111 P 113 P	88	30	157 P 171 P	16.4	0.1	2.4*
2000	0 B 0 B 0 P 0 P	0	0	18 B 34 B 7 P 33 P	23	13	142 P 128 P	13.5	0.0	0.6
4000	B B P P	/	/	B B P P	/	/	97 P 78 P	8.8**	/	/
4-NPDA 0.5	79 94 92 69	84	12	%	/	/	173 164	16.9	3.5*	/
2-AA 3	%	/	/	902 960 821 869	888	58	184 189	18.7	/	24.5*

*: Mutagenic effect
P: Precipitation
**: Bacteriotoxic effect
M: Mean
-S9: without S9 Mix

%: not tested
B: Background lawn reduced

SD: Standard-Deviation
+S9: with S9 Mix

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